

## **Future actions**

### Now

First of all, a huge library of parts could be designed for a construct, which could perform tasks like detection and responding, under specific compositions. It would be good to have a system with different possible combinations which contains wide range of detectors and reporters to enable the systems being applied in several scenarios. Building a combinatorial circuit also implies spending lots of time on construction, under this circumstances, tristable switch could be applied so as to shorten the construction time. Several sets of intermediates with different strengths were prepared for the other teams to use when they need to tune the strength of a system. They could simply use the one with lower activity, for example, weak ribosome binding site together with a repressor gene, if they want to tune the strength down.

Not only could the tristable switch act as a toolkit or cassette to benefit the iGEM teams, but also achieve this with its high specificity and stability. Under no circumstances would the tristable switch be triggered by the other inducers, thus, leakiness could be considerably reduced. Once the system is activated by one of its inducers, the expression would be maintained until the presence of another inducer. Both specificity and stability would be tremendously important for a biosensor. If the sensor fails to fulfil any of these, it would not be able to work in a desirable manner.

With the help from modelling, it would be much easier for iGEM teams to evaluate and apply tristable switch in their project. It is possible for modelling to provide much more information of the system, such as data after the evaluation of a particular composition of parts, once it is implemented with different parts. This kind of prediction would obviously help things go smoother by letting the users

know the basic details, like the properties of some parts combinations, and have a better planning.

### Future

Consisting three different parts, the whole tristable switch could perform three different tasks under the most basic environment sequentially depending on the inducers present. Little modifications would be required to switch its composition. As long as having the fundamental form of the construct, the team would be able to amend its functions according to their needs. Once the tristable switch is further improved with an advanced system, such as the implementation of specific cut site for replacing parts in it and a prediction system provided by modelling, it would be more convenient for the future iGEM teams to use it as a toolkit of construct building and save a lot of time by reducing the time spent on trials and errors for picking the best circuit composition. It would also be easier for iGEM teams to change their construct composites once they realise the mismatch of the promoter and ribosome binding site owing to their strength differences, for example. Various trials and errors and modelling are often involved in this arduous process nowadays which consumes certain amount of time. Applying the tristable switch with the help from modelling in the meantime, the testing time could be reduced by avoiding the difference in the expression level from happening due to the incompatible strength between parts.

With further investigations and advancements of the tristable switch, it is foreseeable that it could really help the iGEM teams in the near future.

### **References**

Giese, B. (Ed.). (2014). *Synthetic Biology: Character and Impact*; Edited by B. Giese, Christian Pade, H. Wigger, Armin Von Gleich. Christian Pade, H. Wigger, Armin Von Gleich. Springer.